Practitioner's Docket No. MPI00-079P1RCP2CN1M

IN THE SPECIFICATION

On page 1, please amend the "Related Applications" paragraph as follows:

This application is a continuation application of U.S. Patent Application Serial No. 09/838,561, filed April 18, 2001; which is a continuation-in-part of U.S. Patent Application Serial No. 09/816,760, filed March 23, 2001, which is a continuation-in-part of U.S. Patent Application Serial No. 09/634,955, filed August 8, 2000, which claims the benefit of U.S. Provisional Application Serial No. 60/192,002, filed March 24, 2000. The entire contents of all of the above-referenced applications are incorporated herein by this reference.

Brief Description of the Drawings

Figures 1A-1D depict the cDNA sequence and predicted amino acid sequence of human DHDR-1 (clone FBH32142). The nucleotide sequence corresponds to nucleic acids 1 to 2660 of SEQ ID NO:1. The amino acid sequence corresponds to amino acids 1 to 802 of SEQ ID NO:2. The coding region without the 3' untranslated region of the human DHDR-1 gene is shown in SEQ ID NO:3.

Figure 2 depicts a structural, hydrophobicity, and antigenicity analysis of the human DHDR-1 protein.

Figure 3 depicts the results of a search which was performed against the MEMSAT database and which resulted in the identification of one "transmembrane domains" in the human DHDR-1 protein (SEQ ID NO:2).

Figure 4 depicts the results of a search which was performed against the HMM database and which resulted in the identification of an "aldehyde dehydrogenase family domain" in the human DHDR-1 protein.

Figures 5A-5B depict the results of a search which was performed against the ProDom database and which resulted in the identification of a "aldehyde dehydrogenase oxidoreductase domain" in the human DHDR-1 protein (SEQ ID NO:2).

Figures 6A-6B depict the cDNA sequence and predicted amino acid sequence of human DHDR-2 (clone Fbh21481). The nucleotide sequence corresponds to nucleic acids 1379 of SEQ ID NO:4. The amino acid sequence corresponds to amino acids 1 to 311 of SEQ ID NO:5. The coding region without the 3' untranslated region of the human DHDR-2 gene is shown in SEQ ID NO:6.

Figure 7 depicts a structural, hydrophobicity, and antigenicity analysis of the human DHDR-2 protein.

Figure 8 depicts the results of a signal peptide prediction and a search which was performed against the MEMSAT database and which resulted in the identification of a signal peptide and one "transmembrane domain" in the human DHDR-2 protein (SEQ ID NO:5).

Figure 9 depicts the results of a search which was performed against the HMM database and which resulted in the identification of a "short-chain dehydrogenase domain" in the human DHDR-2 protein.

Figure 10 depicts the results of a search which was performed against the ProDom database and which resulted in the identification of a "oxidoreductase protein dehydrogenase domain" in the human DHDR-2 protein (SEQ ID NO:5).

Figures 11A-11B depict the cDNA sequence and predicted amino acid sequence of human DHDR-3 (clone Fbh25964). The nucleotide sequence corresponds to nucleic acids 1 to 1725 of SEQ ID NO:7. The amino acid sequence corresponds to amino acids 1 to 369 of

SEQ ID NO:8. The coding region without the 3' untranslated region of the human DHDR-3 gene is shown in SEQ ID NO:9.

Figure 12 depicts a structural, hydrophobicity, and antigenicity analysis of the human DHDR-3 protein.

Figure 13 depicts the results of a search which was performed against the MEMSAT database and which resulted in the identification of four "transmembrane domains" in the human DHDR-3 protein (SEQ ID NO:8).

Figures 14A-14B depicts the results of a search which was performed against the HMM database and which resulted in the identification of a "3-beta hydroxysteroid dehydrogenase domain", a "short chain dehydrogenase domain", and a "NAD-dependent epimerase/dehydratase domain" in the human DHDR-3 protein.

Figure 15 depicts the results of a search which was performed against the ProDom database and which resulted in the identification of a "3-beta hydroxysteroid dehydrogenase domain" in the human DHDR-3 protein (SEQ ID NO:8).

Figure 16 depicts the cDNA sequence and predicted amino acid sequence of human DHDR-4 (clone Fbh21686). The nucleotide sequence corresponds to nucleic acids 1 to 1209 of SEQ ID NO:10. The amino acid sequence corresponds to amino acids 1 to 322 of SEQ ID NO:11. The coding region without the 3' untranslated region of the human DHDR-4 gene is shown in SEQ ID NO:12.

Figure 17 depicts an alignment of the human DHDR-4 amino acid sequence ("21686"; SEQ ID NO:11) with the amino acid sequence of *Rattus norvegicus* putative short-chain dehydrogenase/reductase ("5052204_SDR_rat"; GenBank Accession Number AF099742; SEQ ID NO:13) using the CLUSTAL W (1.74) multiple sequence alignment program. Identical amino acids are indicated by stars.

Figure 18 depicts a structural, hydrophobicity, and antigenicity analysis of the human DHDR-4 protein.

Figure 19 depicts the results of a signal peptide prediction and a search which was performed against the MEMSAT database and which resulted in the identification of a "signal peptide" and four "transmembrane domains" in the human DHDR-4 protein (SEQ ID NO:11).

Figure 20 depicts the results of a search which was performed against the HMM database and which resulted in the identification of a "short chain dehydrogenase domain" and a "short chain dehydrogenase/reductase domain" in the human DHDR-4 protein.

Figures 21A-21B depict the results of a search which was performed against the ProDom database and which resulted in the identification of a "oxidoreductase protein dehydrogenase domain", a "shikimate 5-dehydrogenase domain", a "dehydrogenase domain" and a "glucose-1-dehydrogenase domain" in the human DHDR-4 protein (SEQ ID NO:11).

Figure 22 depicts the expression levels of human DHDR-1 mRNA in various human cell types and tissues, as determined by Taqman analysis. Samples: (1) normal artery; (2) normal vein; (3) aortic smooth muscle cells - early; (4) coronary smooth muscle cells; (5) human microvascular endothelial cells (HMVECs) - static; (6) human microvascular endothelial cells (HMVECs) - shear; (7) normal heart; (8) heart - congestive heart failure (CHF); (9) kidney; (10) skeletal muscle; (11) normal adipose tissue; (12) pancreas; (13) primary osteoblasts; (14) differentiated osteoclasts; (15) normal skin; (16) normal spinal cord; (17) normal brain cortex; (18) brain - hypothalamus; (19) nerve; (20) dorsal root ganglion (DRG); (21) resting peripheral blood mononuclear cells (PBMCs); (22) glioblastoma; (23) normal breast; (24) breast tumor; (25) normal ovary; (26) ovary tumor; (27) normal prostate; (28) prostate tumor; (29) epithelial cells (prostate); (30) normal colon; (31) colon tumor; (32) normal lung; (33) lung tumor; (34) lung - chronic obstructive pulmonary disease (COPD); (35) colon - inflammatory bowel disease (IBD); (36) normal liver; (37) liver - fibrosis; (38) dermal cells - fibroblasts; (39) normal tonsil; (40) lymph node; (41) small intestine; (42) skin - decubitus; (43) synovium; (44) bone marrow mononuclear cells (BM-MNC); (45) activated peripheral blood mononuclear cells (PBMCs).

Figure 23 depicts the expression levels of human DHDR-1 mRNA in various types of human tumors, as determined by Taqman analysis. Samples: (1-3) normal breast; (4) breast tumor - infiltrating ductal carcinoma (IDC); (5) breast tumor - infiltrating ductal carcinoma (MD-IDC); (6-8) breast tumor - infiltrating ductal carcinoma (IDC); (9) breast tumor; (10-11) normal ovary; (12-16) ovary tumor; (17-19) normal lung; (20) lung tumor - SmC; (21-23) lung tumor - poorly differentiated non-small cell carcinoma of the lung (PDNSCCL); (24) lung tumor - small cell carcinoma (SCC); (25) lung tumor - AC; (26) lung tumor - ACA; (27-29) normal colon; (30-31) colon tumor - MD; (32) colon tumor; (33) colon tumor - MD-PD; (34-35) colon tumor - liver metastasis; (36) normal liver (female); (37) hemangioma; (38) human microvascular endothelial cells (HMVECs) - arrested; (39) human microvascular endothelial cells (HMVECs) - proliferating.

Figure 24 depicts the expression levels of human DHDR-1 mRNA in various human colon tumor samples, as determined by Taqman analysis. Samples: (1-6) normal colon; (7-8) adenomas; (9-15) colonic ACA-B; (16-21); colonic ACA-C; (22-27) normal liver; (28-33) colon tumor - liver metastasis; (34) colon tumor - abdominal metastasis.

Figure 25 depicts the expression levels of human DHDR-1 mRNA in NOC synchronized HCT116 cells at various time points after entry into the cell cycle, as determined by Taqman analysis. The time point t = 0 signifies the G2/M border. Samples: (1) t = 0; (2) t = 3; (3) t = 6; (4) t = 9; (5) t = 15; (6) t = 18; (7) t = 21; (8) t = 24.

Figure 26 depicts the expression levels of human DHDR-2 mRNA in various human clinical tumor samples, as determined by Taqman analysis. Samples: (1-4) normal breast;

(5-11) breast tumor; (12-14) normal lung; (15-22) lung tumor; (23-25) normal colon; (26-33) colon tumor; (34-37) colon tumor - liver metastasis; (38-39) normal liver; (40) normal brain; (41-43) brain tumor - glioblastoma.

Figure 27 depicts the expression levels of human DHDR-4 mRNA in various human cell types and tumors, as determined by Taqman analysis. Samples: (1) normal aorta; (2) normal fetal heart; (3) normal heart; (4) heart - congestive heart failure (CHF); (5) normal vein; (6) aortic smooth muscle cells; (7) normal spinal cord; (8) normal brain cortex; (9) brain - hypothalamus; (10) glial cells - astrocytes; (11) brain - glioblastoma; (12) normal breast; (13) breast tumor - infiltrating ductal carcinoma (IDC); (14) normal ovary; (15) ovary tumor; (16) pancreas; (17) normal prostate; (18) prostate tumor; (19) normal colon; (20) colon tumor; (21) colon - inflammatory bowel disease (IBD); (22) normal kidney; (23) normal liver; (24) liver - fibrosis; (25) normal fetal liver; (26) normal lung; (27) lung tumor; (28) lung - chronic obstructive pulmonary disease (COPD); (29) normal spleen; (30) normal tonsil; (31) normal lymph node; (32) normal thymus; (33) epithelial cells - prostate; (34) endothelial cells - aortic; (35) skeletal muscle; (36) dermal fibroblasts; (37) normal skin; (38) normal adipose tissue; (39) primary osteoblasts; (40) undifferentiated osteoblasts; (41) differentiated osteoblasts; (42) osteoclasts; (43) aortic smooth muscle cells (SMCs) - early; (44) aortic smooth muscle cells (SMCs) - late; (45) human umbilical vein endothelial cells (HUVECs) - shear; (46) human umbilical vein endothelial cells (HUVECs) - static.

Figure 28 depicts the expression levels of human DHDR-4 mRNA in various cell types and tissues, as determined by Taqman analysis. Samples: (1-2) normal liver; (3-4) HBV+ liver; (5) HCV+ liver; (6) HepG2-B cells; (7) HepG2.2.15-B cells; (8) HepG2 cells (no treatment); (9) HepG2 cells - treated with Bayer compound (IC50); (10) HepG2 cells - treated with Bayer compound (IC100); (11) HepG2.2.15 cells (no treatment); (12) HepG2.2.15 cells - treated with Bayer compound (IC50); (13) HepG2.2.15 cells - treated with Bayer compound (IC100); (14) HepG2 control; (15) HepG2 cells transfected with the HBV-X gene; (16) HuH7 cells; (17-19) ganglia; (20) NT2/KOS - 0 hr.; (21) NT2/KOS - 2.5 hr.; (22) NT2/KOS - 5 hr.; (23) NT2/KOS - 7 hr.; (24) MRC/VZV - mock; (25) MRC/VZV - 18 hr.; (26) MRC/VZV - 72 hr.

Figure 29 depicts the expression levels of human DHDR-4 mRNA in various human tumor samples, as determined by Taqman analysis. Samples: (1-4) normal colon; (5-11) colon tumor; (12-15) colon tumor - liver metastasis; (16-17) normal liver; (18-21) normal brain; (22-27) brain tumor - glioblastoma; (28-29) human microvascular endothelial cells (HMVECs); (30) placenta; (31-32) fetal adrenal gland; (33-34) fetal liver.

Figure 30 depicts the expression levels of human DHDR-4 mRNA in various human tumors and synchronized A549 cells at various time points after entry into the cell cycle, as determined by Taqman analysis. The time point t = 0 signifies the G2/M border. Samples: (1-4) normal breast; (5-10) breast tumor; (11-14) normal ovary; (15-22) ovary tumor; (23-

26) normal lung; (27-34) lung tumor; (35-42) synchronized A549 cells: (35) t = 0; (36) t = 3 (RT); (37) t = 3 (-RT); (38) t = 6; (39) t = 9; (40) t = 12 (RT); (41) t = 18; (42) t =24.

Figures 31A-31B depict the cDNA sequence and predicted amino acid sequence of mouse DHDR-2 (clone m21481). The nucleotide sequence, corresponding to nucleic acids 1 to 1108 of SEQ ID NO:14, is shown in *Figure 31A*. The coding region, corresponding to SEQ ID NO:16, is underlined. The amino acid sequence, corresponding to amino acids 1 to 311 of SEQ ID NO:15, is shown in *Figure 31B*.

Figures 32A-32B depict an alignment of the mouse DHDR-2 nucleotide sequence ("M21484"; SEQ ID NO:14) with the human DHDR-2 nucleotide sequence ("h21484"; SEQ ID NO:4) using the GAP program in the GCG software package (nwsgapdna.cmp matrix) and a gap weight of 12 and a length weight of 4. As shown in the alignment, the mouse and human DHDR-2 nucleotide sequences are about 88.1% identical.

Figure 33 depicts an alignment of the mouse DHDR-2 amino acid sequence ("m21484"; SEQ ID NO:15) with the human DHDR-2 amino acid sequence ("h21484"; SEQ ID NO:5) using the GAP program in the GCG software package (Blosum 62 matrix) and a gap weight of 12 and a length weight of 4. As shown in the alignment, the mouse and human DHDR-2 amino acid sequences are about 91.3% identical.

Detailed Description of the Invention

The present invention is based, at least in part, on the discovery of novel molecules, referred to herein as "dehydrogenase" or "DHDR" nucleic acid and protein molecules, which are novel members of a family of enzymes possessing dehydrogenase activity. These novel molecules are capable of oxidizing or reducing biological molecules by catalyzing the transfer of a hydride moiety and, thus, play a role in or function in a variety of cellular processes, *e.g.*, proliferation, growth, differentiation, migration, immune responses, hormonal responses, inter- or intra-cellular communication, and viral infection.

As used herein, the term "dehydrogenase" includes a molecule which is involved in the oxidation or reduction of a biochemical molecule (*e.g.*, an amino acid, a vitamin, a steroid such as a glucocorticoid, or a nucleic acid), by catalyzing the transfer of a hydride ion to or from the biochemical molecule. Dehydrogenase molecules are involved in the metabolism and catabolism of biochemical molecules necessary for energy production or storage, for intra- or inter-cellular signaling, for metabolism or catabolism of metabolically important biomolecules, and for detoxification of potentially harmful compounds. Examples of dehydrogenases include alcohol dehydrogenases, aldehyde dehydrogenases, steroid dehydrogenases, and lipid dehydrogenases. Thus, the DHDR molecules of the present invention provide novel diagnostic targets and therapeutic agents to control dehydrogenase-associated disorders.

and homologues of the DHDR cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the DHDR gene.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 15, 20, 25, 30 or more nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 14, or 16, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, PTA-1845, _____, _____, or _____. In other embodiment, the nucleic acid is at least 50-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-550, 550-600, 600-650, 650-700, 700-750, 750-800, 800-850, 850-900, 900-950, 950-1000, 1000-1050, 1050-1100, 1100-1150, 1150-1200, 1200-1250, 1250-1300, 1300-1350, 1350-1400, 1400-1450, 1450-1500, 1500-1550, 1550-1600, 1600-1650, 1650-1700, 1700-1750, 1750-1800, 1800-1850, 1850-1900, 1900-1950, 1950-2000 or more nucleotides in length.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4, and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9, and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or alternatively hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or alternatively hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1X SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less

than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6($\log_{10}[Na^+]$) + 0.41(%G+C) - (600/N), where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1X SSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C (see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995), or alternatively 0.2X SSC, 1% SDS.

Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 14, or 16, and corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In addition to naturally-occurring allelic variants of the DHDR sequences that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into the nucleotide sequences of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 14 or 16, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, PTA-1845, _____, _____, or _____, thereby leading to changes in the amino acid sequence of the encoded DHDR proteins, without altering the functional ability of the DHDR proteins. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence of SEQ ID NO:1, 3, 4, 6, 7, 9, 10, 12, 14, or 16, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number _____, PTA-1845, _____, _____, or _____. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of DHDR (*e.g.*, the sequence of SEQ ID NO:2, 5, 8, 11, or 15) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the DHDR proteins of the present invention, *e.g.*, those present in a transmembrane domain, are predicted to be particularly unamenable to alteration. Furthermore, additional amino acid residues that are conserved between the DHDR proteins of the present invention and other members of the DHDR family are not likely to be amenable to alteration.